SEDIMENT TOXICITY OF LONG MEADOW LAKE MINNESOTA VALLEY NATIONAL WILDLIFE REFUGE

by

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Project ID #90-3-108

Completion Report to the
U.S. Fish and Wildlife Service
Office of Environmental Contaminants
Federal Building, Fort Snelling
Twin Cities, MN 55111

December 1992

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ABSTRACT

Long Meadow Lake on the Minnesota Valley National Wildlife Refuge - an important waterfowl production area - serves as a major urban stormwater receptor in the southern Twin Cities Metropolitan Area. Refuge concerns that contamination associated with urban runoff to the lake may be impairing its invertebrate productivity prompted the present investigation of the toxicity of contaminated lake bed sediments in the outfall zone of a major urban runoff collection system. In 1990, sediment from that area ("treatment" site) and from a mid-lake reference site underwent laboratory analyses for heavy metals and polynuclear aromatic hydrocarbons. Larval chironomid (*Chironomus tentans*) and burrowing mayfly (*Hexagenia limbata*) partial life cycle tests were performed on bulk sediment, sediment pore water and sediment elutriate from both sites. MICROTOX® tests were performed on pore water and elutriate.

Test results for all three sediment phase exposures showed varying levels of toxicity-induced mortality and weight reduction compared to controls. Pore water was generally the most toxic phase tested and bulk sediment the least toxic. While the test organisms' response to even the treatment sediment was not dramatic, the analytical data suggest that other, more contaminated sediment may exist in the stormwater outfall zone. Use of MICROTOX® to screen sediments over a broader area of the outfall zone possibly followed by additional invertebrate toxicity testing of those sediments showing the greatest screening response is recommended.

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INTRODUCTION

Long Meadow Lake in the Minnesota Valley National Wildlife Refuge (Refuge) is an important mallard and wood duck production area, but Refuge biologists have suspected that the lake has not produced waterfowl broods commensurate with its apparent capacity. This lower than expected level of waterfowl production, combined with the fact that Long Meadow Lake is the only lake within the Refuge to receive large volumes of urban stormwater runoff, prompted Refuge and contaminants biologists to investigate the possibility of a contaminant-related problem.

In 1988, surficial sediments near the mouths of nine urban stormwater outfalls to Long Meadow Lake were collected and analyzed for heavy metals and polynuclear aromatic hydrocarbons (PAHs). Six sediment samples obtained at varying distances lakeward of the Pond C stormwater outfall were found to contain substantially elevated sediment contaminant concentrations relative to the others (lead: 155 to 438 ppm dry weight, copper: 47 to 94 ppm, nickel: 16 to 30 ppm, chromium: 19 to 41 ppm, zinc: 171 to 349 ppm and total PAHs: 15 to 44 ppm). While such a sediment environment in a refuge waterbody is intuitively undesirable, there is little or no information available relating complex sediment contaminant mixtures to acute or chronic toxicity to sediment-dwelling aquatic life.

The present study, conducted in 1990, involved the chemical analysis of sediments obtained from the Pond C outfall zone in Long Meadow Lake and from a mid-lake "reference" site coupled with the exposure of MICROTOX® bioluminescent bacteria and two sensitive benthic invertebrate species to those sediments and to the available contaminant fraction present in their interstitial waters ("pore water" and "elutriate"). The intent of the study was to determine the aquatic toxicity of a relatively strong sediment mix of typical urban runoff chemical constituents in order to provide the Refuge Manager with a perspective from which to develop positions relative to current or future expanded stormwater inputs to Long Meadow Lake or other Refuge waters.

METHODS

In July 1990, Pond C outfall test sediment was obtained approximately 70 feet lakeward of the emergent vegetation line near the outfall channel in an area previously showing high sediment contaminant concentrations. "Reference" sediment -intended to represent the general offshore sediment contaminant condition - was obtained near mid-lake approximately 100 yards east of the new Cedar Avenue bridge. Sediments from each site were sent to Service contract laboratories for heavy metals, PAHs, and particle size analysis. Approximately one gallon of additional material from each site was delivered to the University of Minnesota Cooperative Fish and Wildlife Research Unit for use in toxicity testing.

Three sediment phases were tested: bulk sediment, sediment elutriate, and sediment pore water. Two benthic invertebrates, chironomids (*Chironomus tentans*) and burrowing mayflies *(Hexagenia limbata)*, were evaluated for toxic response. In addition to these six toxicity tests (3 phases x 2 invertebrate species), a microbial toxicity test, MICROTOX®, was also conducted using elutriate and pore-water.

The benthic invertebrates used in these toxicity tests were chosen for several reasons. Both *H. limbata* and *C. tentans* are indigenous to the Long Meadow Lake area, and both are important components of fish and waterfowl diets. Additionally, both organisms are truly benthic, enhancing the probability that observed toxic responses are due to sediment-associated contamination. Finally, *C. tentans* and *H. limbata* are both important indicators of ecosystem health and have been shown to be sensitive to environmental contaminants (Cairns et al., 1984).

I. TEST MATERIAL HANDLING AND PHASE PREPARATION

A. Bulk Sediment

To directly determine the bioavailability and toxicity of sediment-bound contaminants, bulk sediment toxicity tests were performed. Unmodified, field-collected sediments were stored at 4 degrees Centigrade (C) for seven days until animal sorting and test set-up was completed so that data collection could begin.

B. Sediment Elutriate

Elutriates prepared from sediments (by mixing clean water with contaminated sediment) were used to isolate and concentrate water soluble contaminants associated with bulk sediments. Those fractions are released when the sediment is disturbed and resuspended in the water column (Daniels et al., 1989). The elutriate was prepared using a modified version of the Standard Elutriate Test (USEPA and USACE, 1991). To produce the recommended 4:1 ratio of well water and sediment, one liter (L) of well water was mixed with 250 milliliters (ml) of sediment. The mixture was shaken using a variable speed shaker at a rate of 100 exchanges per minute for 30 minutes and allowed to settle for one hour. The mixture was poured into 250 ml containers and

centrifuged at a relative centrifugal force (RCF) of 5,000 for 10 minutes. The supernatant was then transferred into one L containers and stored at 4 degrees C until toxicity tests were begun 24 hours later.

C. Pore water Preparation

Sediment pore water - the water occupying the interstitial spaces between sediment particles - was extracted from bulk sediment and used as an indicator of the potential toxicity of the sediment. Multiple 300 gram bulk sediment subsamples were centrifuged at 5,000 RCF for 45 minutes in 250 ml polypropylene containers (Giesy et al., 1988). The supernatant was filtered using a 250 micron Nitex® screen. Pore water was stored at 4 degrees C until used for toxicity tests, which occurred within 7 days.

II. TOXICITY TESTING

All toxicity tests were initially performed using full strength, field-collected bulk sediment, laboratory-prepared elutriate and pore water. If toxicity was detected in a full strength sample, then a dilution series would have been prepared to determine the magnitude of the toxicity. This dilution preparation step was not found to be necessary.

A. MICROTOX®

The MICROTOX® toxicity tests utilized rehydrated lyophilized cells of *Photobacterium phosphoreum*, a luminescent bacteria. The light producing mechanism of *P. phosphoreum* is normal and directly related to the metabolism of the bacteria. If the organisms' metabolic processes are disrupted due to exposure to toxicants, a corresponding inhibition of light production will occur and can be measured. This change in light production can be compared to light production by unexposed bacteria, and quantification along with calculation of lethal concentrations is possible. Both elutriate and pore water samples were examined using MICROTOX®. In both cases, the toxicity tests were performed according to the 100% Assay Procedure (Microbics, Inc., 1989).

B. CHIRONOMID EXPOSURES

Egg cases of *C. tentans* from University of Minnesota Cooperative Fish and Wildlife Research Unit cultures were placed in blended paper towel substrate and allowed to develop to the second instar, 14-16 days post-hatch. These second instar larvae were used in 14-day toxicity tests (Mosher et al., 1982) to evaluate bulk sediment, elutriate, and pore water toxicity. Individual 50 ml polypropylene centrifuge tubes were used as test chambers for single individuals so that molting frequency could be followed and recorded. Each treatment group or site examined had 15 replicates, one larvae per chamber.

Bulk sediment test chambers (glass jars, appropriately cleaned) containing 7.5 grams of test sediment were filled to the 50 ml mark with well water, achieving the 4:1 water to sediment ratio previously mentioned. A second set of chambers contained 7.5 grams of either chironomid culture medium or reference site sediment.

The elutriate and pore water test chambers contained 7.5 grams of blended paper towel substrate. The extracted pore water and elutriate was evenly divided between the replicate test chambers for each treatment group. This division resulted in elutriate and pore water test chamber volumes of 45 ml per chamber. The control test chambers for the elutriate and pore water tests contained 7.5 grams of blended paper towel substrate and were filled to the 45 ml mark with well water in which the organisms were cultured.

Continual aeration was supplied to each test chamber, and the larvae were fed 0.1 ml of food daily. Water temperature and mortality (if observed) were recorded daily. When the test was terminated, the larvae were placed in aluminum ashing pans, dried in an 80 degree C oven for 24 hours, and weighed to the nearest 0.1 mg. A reduction in weight gain relative to control organisms was calculated. To avoid underestimating the effects of sediment toxicity, the reduction of weight gain relative to control organisms was defined as 100% for individuals that died during exposure (Giesy et al., 1988).

C. *Hexagenia limbata* Exposure

H. limbata nymphs were acclimated to test water in the laboratory for 48 hours prior to testing. Acute, static and 10-day *H. limbata* toxicity tests (Nebecker et al., 1984 and Fremling et al., 1980) were used to assess the potential toxicity of the bulk sediment, elutriate, and pore-water. The 4 ounce, straight walled, glass jars used as test chambers were acid washed and acetone rinsed. Each treatment group consisted of 10 replicated test chambers. Each test chamber contained one nymph.

The bulk sediment test chambers contained 50 grams of contaminated sediment and 100 ml of overlying well water. The control chambers for the bulk sediment test contained 50 grams of control sediment and 100 ml well water.

The elutriate and pore water test chambers each contained 100 ml of aqueous phase solution. Artificial, stainless steel burrows were placed in the elutriate and pore water test chambers to accommodate the thigmotactic requirements of *H. limbata*. Control chambers contained 100 ml of well water and a stainless steel burrow.

All test chambers were fitted with stoppers, wrapped in acetone-rinsed aluminum foil, and aerated. Test chambers were held in a flow-through water bath at 18 degrees C under low light conditions. Nymphs were fed 0.2 ml of a prepared diet every other day. The measured endpoints were lethality and molting frequency as a reflection of growth; therefore, mortality and number of molts were recorded daily.

RESULTS AND DISCUSSION

I. ANALYTICAL CHEMISTRY RESULTS

Results of heavy metal, PAH, and particle size analyses are provided in the following Table. In the present study, contaminant concentrations in the Pond C outfall test sediment were, on average, considerably higher than those of the mid-lake "reference" site. However, concentrations of some of the more toxic metals in the test sediment were substantially lower than those found in sediments from the same general area during the 1988 sediment survey. That fact, coupled with the relatively high percentage of sand-size particles in the test sample, suggests that toxicity testing was not performed on the most contaminated sediments existing within the Pond C outfall zone.

II. TOXICITY TESTS

A. MICROTOX®

The MICROTOX® toxicities of water extracted from sediment from the two Long Meadow Lake sites are shown in Figure 1. The toxicities of sediment elutriate and pore water are expressed as the percentage of full-strength solution eliciting a 50% light attenuation response (EC50) from the test bacteria. Site LML-T-PW (Pond C outfall pore water treatment site) had the lowest 15-minute EC50 of 79.78%. LML-T-PW was the only sediment-derived solution to induce a MICROTOX® response.

B. *Chironomus tentans*

The results of the *C. tentans* toxicity tests are displayed in Figures 2 and 3. Figure 2 shows the percent larval weight reduction after exposures to elutriate, pore water and sediment from both Long Meadow Lake sediments relative to a control. Figure 3 displays the percent mortality observed after exposure to elutriate, pore water and sediment from those same sources. Some evidence of a toxic response was noted in chironomids exposed to all three sediment phases at both Long Meadow Lake sites. The percent weight reduction of *C. tentans* in the elutriate phase from sites LML-T and LML-R (Long Meadow Lake reference site) was significantly greater than the control. A significant reduction in weight was also detected in chironomids exposed to pore water extracted from site LML-T. Exposure to bulk sediment from either site produced no significant weight reduction in *C. tentans*.

C. *Hexagenia limbata*

The percent mortality for sites LML-T and LML-R is displayed in Figure 4. Elutriate phase testing resulted in 10% mortality in LML-T with no mortality observed in elutriate from site LML-R. Pore-water testing from LML-T and LML-R yielded 40% and 30% mortality, respectively. Bulk sediment exposure showed no mortality associated with LML-T samples, while 30% mortality was observed in LML-R samples.

CONCLUSIONS

The toxicity test results from elutriate, pore water and bulk sediment exposures all showed marginal levels of toxicity-induced mortality. *C. tentans* pore water exposures produced the highest percent body weight reduction. Pore water, in general, seemed to be the most toxic phase tested, showing a trend of higher mortality and toxic response throughout the evaluation and across the species tested. However, site LML-R elutriate produced a higher percent mortality and weight reduction of *C. tentans* than the pore water from this site. While Site LML-T did show slightly higher mortality and weight reduction and lower EC50 values than site LML-R, the difference was not statistically significant. Bulk sediment exposure tests revealed no significant contaminant-related effect to either of the test organisms.

Based on results of the chemical analyses performed, the limited toxicity observed in this study is likely related to individual or collective concentrations of the heavy metals and PAHs found in the test sediments. Chemical and toxicological results, when interpreted together, can sometimes provide a better understanding of contaminant-related cause-effect relationships in aquatic systems. From the data collected in this study, however, only slight toxicity was induced by sediment-associated water soluble contaminants collected from both sites LML-T and LML-R. Bulk sediment results indicated no significant toxicity. This negative finding may be related, in part, to the fact that testing was performed on surficial sediments which clearly were not as contaminated as those found during a 1988 survey of the outfall area.

RECOMMENDATION

In order to further examine the possibility that surficial sediments located elsewhere within the Pond C outfall zone in Long Meadow Lake may be more toxic to aquatic life, the Twin Cities Field Office (TCFO) Contaminants staff would be willing to undertake a more thorough screening of sediment pore waters obtained from that area. Our Contaminants staff has MICROTOX® capability, which can be used to identify zones or pockets of relatively greater toxicity - if they exist - for possible followup invertebrate toxicity testing similar to that described for the present study. Workload scheduling requires that such a study be delayed until at least FY 94. You will be contacted during FY 93 to determine your interest in supporting such a study.

TABLES

Table. Selected heavy metal and polynuclear aromatic hydrocarbon concentrations, and percent sand, silt, clay in sediments from relatively contaminated (Pond C urban stormwater discharge zone) and uncontaminated (mid-lake) sites in Long Meadow Lake, Minnesota Valley National Wildlife Refuge.

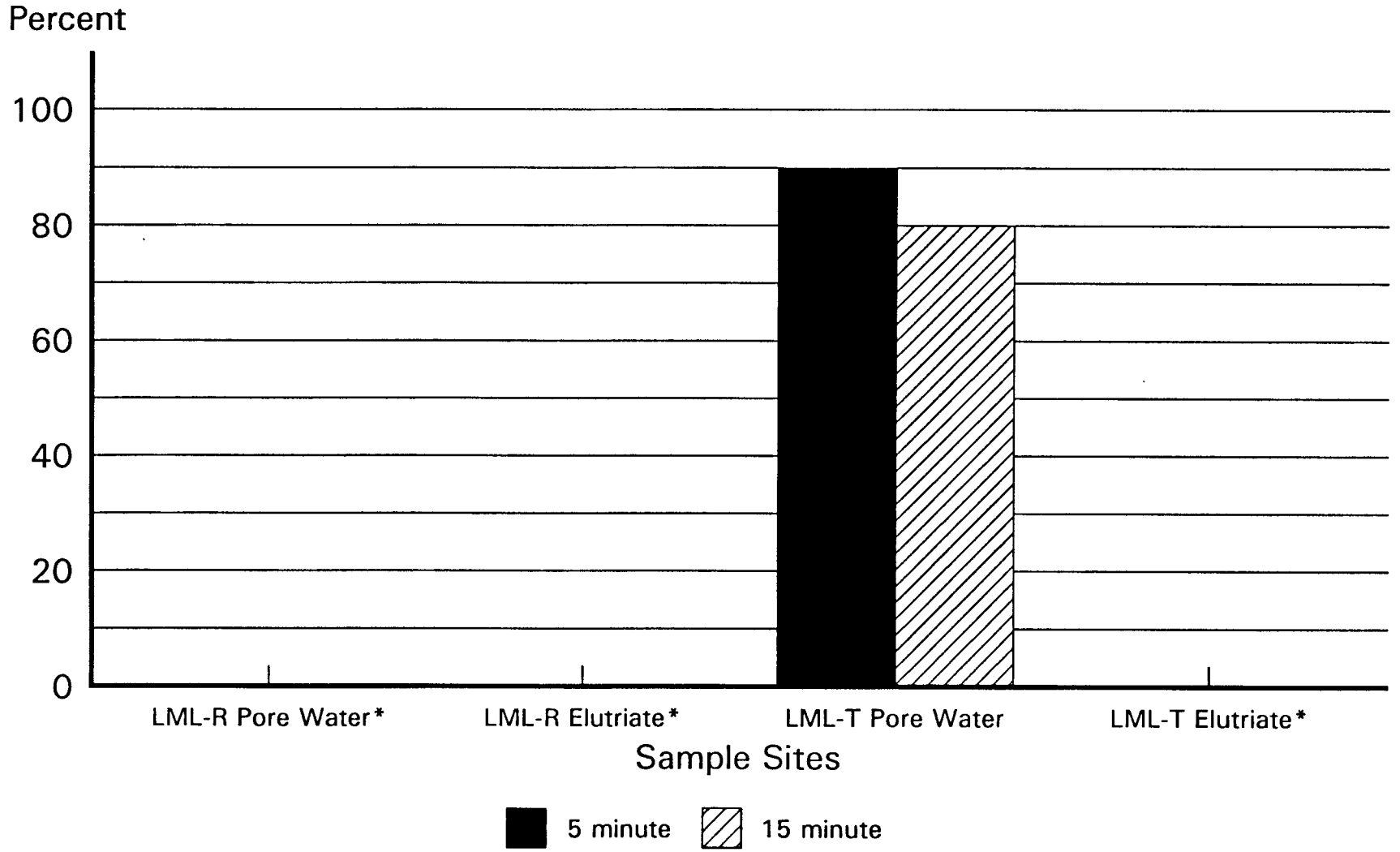
Sediment Concentrations (ppm Dry Weight)			
	Pond C Discharge Zone		
Parameter	1988 Survey	Present Study	Mid-lake
Aluminum	5,760-12,800	13,600	11,300
Arsenic	7.5-11.8	10	<10
Cadmium	BDL	1.9	0.7
Chromium	19.2-41.5	33	20
Copper	48.7-94.3	49.5	22.3
Iron	13,800-23,700	21,800	19,500
Manganese	738-1,250	1,080	470
Nickel	15.9-30.7	28	21
Lead	155-438	280	68
Zinc	171-349	282	94.1
Total PAHs	15.0-43.8	47.1	3.04
Particle size			
(% Sand)	NA	18.6	7.1
(% Silt)	NA	66.6	61.7
(% Clay)	NA	14.8	31.2

BDL = Below Detection Limit

NA = Not Analyzed

FIGURES

Fig. 1. Percent of full strength sediment elutriate and pore water from sites LML-T and LML-R producing MICROTOX EC50 response



* Full strength solution produced no MICROTOX response

Fig. 2. Percent weight reduction in C. tentans exposed 14 days to LML-T and LML-R sediment, elutriate and pore water compared to control

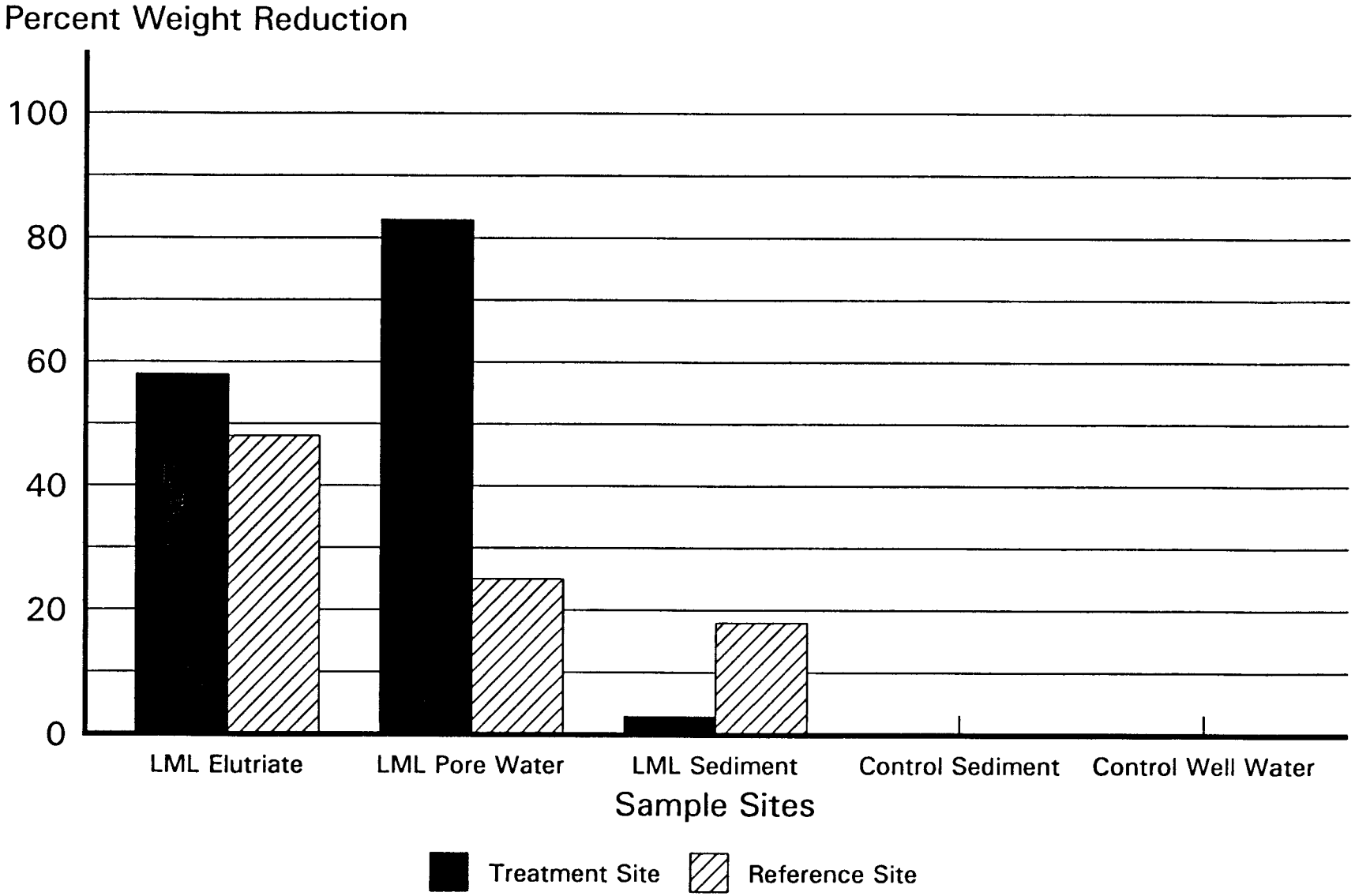


Fig. 3. Percent mortality in C. tentans exposed 14 days to LML-T and LML-R bulk sediment, elutriate and pore water compared to control

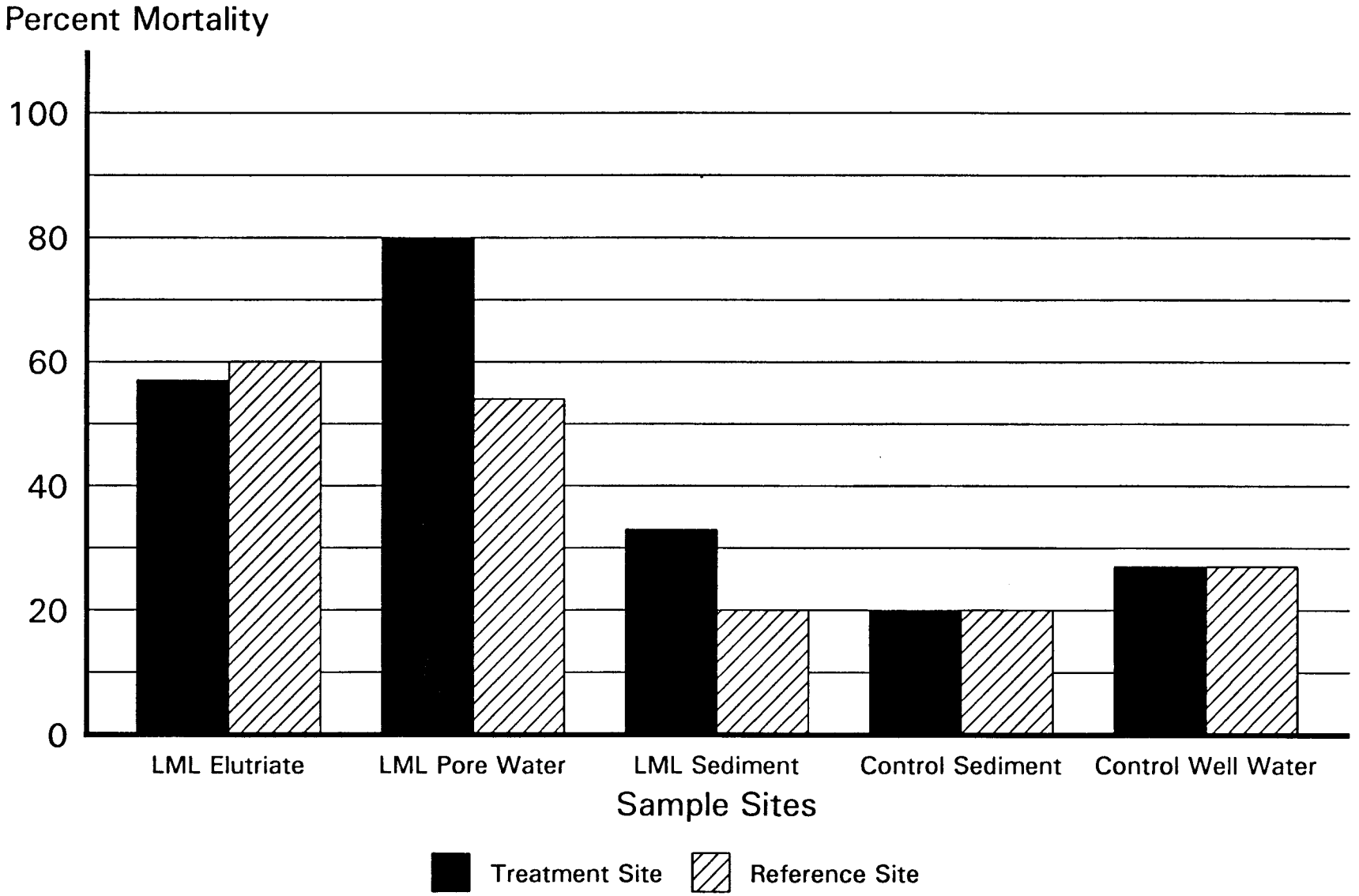
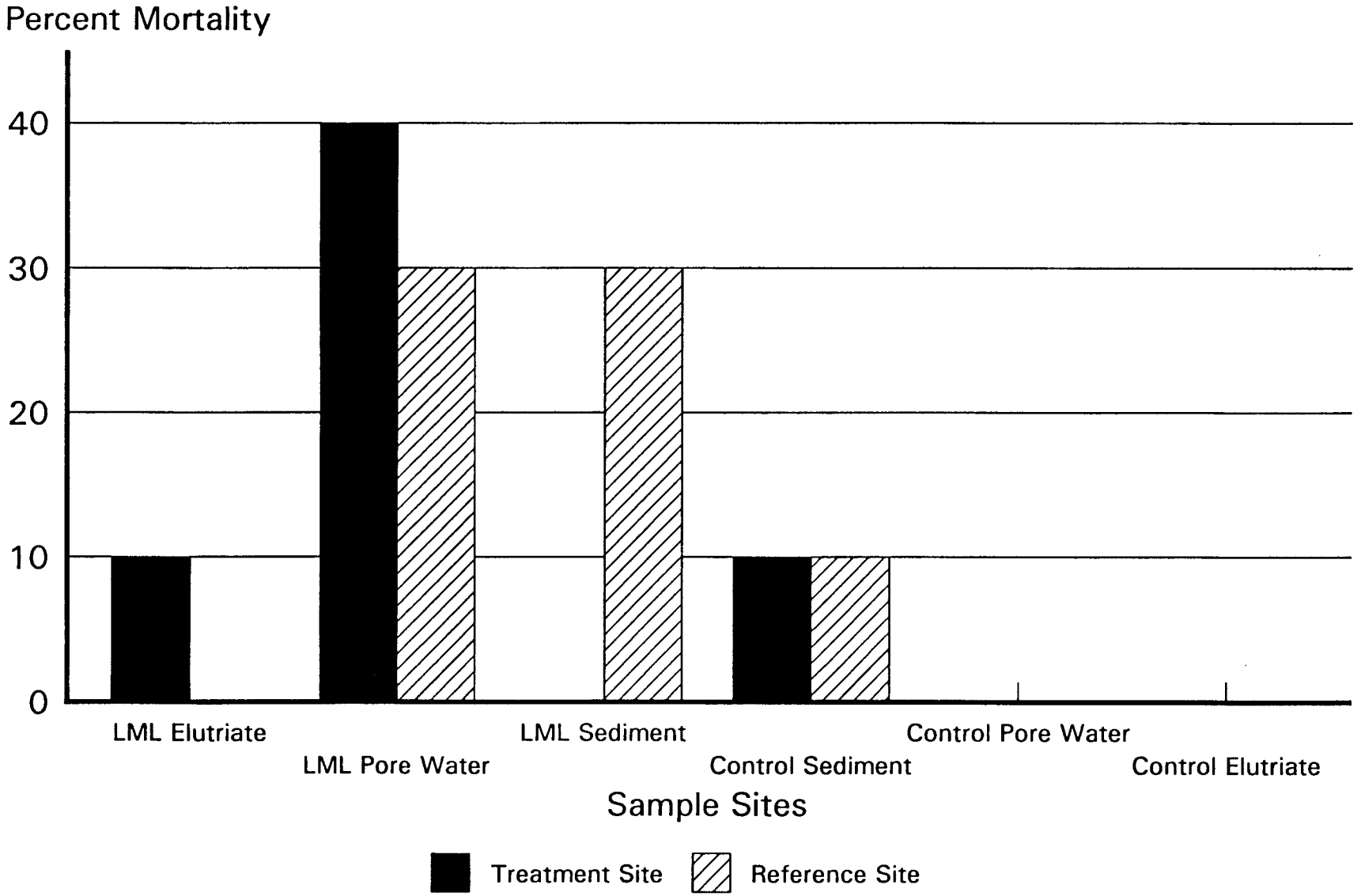


Fig.4. Percent mortality of Hexagenia limbata exposed 14 days to LML-T and LML-R sediment, elutriate and pore water compared to control



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